VIRUS, Previous Infection

#532
Cf. #362

Activated: 5/1/63
Renewed: 5/1/64
Renewed: 5/1/65

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

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COMMITTEE:
Dr. Jacobson, Chm.
Dr. Lynch
Dr. Little

Application For Research Grant

Date: January 31, 1966

1. Name of Investigator: JOHN P. MANOS, M.D.

2. Title: Associate in Microbiology

3. Institution & Address: MEDICAL COLLEGE OF SOUTH CAROLINA
80 Barre Street
Charleston, South Carolina 29401

4. Project or Subject: THE ANTIGENIC STUDY OF THE VARIOUS NEOPLASTIC SUBCELLULAR PARTICLES FOLLOWING SEPARATION BY ULTRA-FILTRATION METHODS.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

That neoplastic cells hava an antigenic makeup distinct at least in part from normal cells has been a source of much interest and investigation. The significance of such studies for potential diagnostic, therapeutic, preventive and etiologic evaluations is obvious. Some pertinent references are listed.

As an outgrowth of a project currently in progress, it was felt that an antigenic study of subcellular fractions of neoplastic cells by methods used in the other project, i.e. differential ultra-filtration, the emergence of specific 'cancer' antigens might be effected. As indicated in progress report #4, work has been begun in this area. Cells from a human lung cancer and normal human lung have been disrupted mechanically by grinding and the sub-cellular material fractionated by ultra-filtration methods using the Gelman filterfuge apparatus.

These fractions, 10 from the human lung carcinoma and 10 from normal human lung, have been inoculated in paired rabbits using Freund's complete adjuvant and an intensive immunization schedule. The rabbits were exsanguinated and the cell fractions are being tested for antigenicity. The first method of approach is that of gel double immunodiffusion. Preliminary work by this method has shown that some of the fractions so far tested, both from cancer and normal lung cell are antigenic. It is planned, by cross absorption techniques to determine if any of the cancer cell fractions have or lack antigenic component(s) absent or present in the normal cell fractions. Other methods to be used for determining antigen specificity are the tanned red cell and possibly the complement-fixation techniques. Also, other approaches to the preparation of fractions could be used, such as density gradient centrifugation and column chromatography.

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Zibler, L. A., Study of the Tumor Specificity of Gastric Cancer Tissue. Ann. N. Y. Acad. Sci., 101(1):264, 1962

6. Budget Plan:

a.	Salaries	-
b.	Expendable Supplies	5000.00
c.	Other Expenses	1000.00
d.	Permanent Equipment	3000.00
e.	Overhead (15% of a, b, c)	900.00
	Total	9900.00

7. Anticipated Duration of Work:

3 Years

8. Facilities and Staff Available:

Millipore filter apparatus and filters, Gelman centrifuge filter holders and filter, various centrifuges, including the Serval Regrigerated super-speed centrifuge, spectronhotometers, Micro Kjeldahl equipment, photographic equipment, chromatographic apparatus, sonic oscillators, fluorescent microscopy equipment, electron microscopy and other equipment pertinent to microbiological work. Apparatus for double immunodiffusion techniques and other serological techniques. Column chromatographic apparatus.

9. Additional Requirements: Need for a preparative ultra-centrifuge in the future.

10. Additional Information (Including relation of work to other projects and other sources of support):

The methods used for subcellular fraction preparation derived from current project studying similar tyne fractions from mouse lung adenomas in an attempt to demonstrate viral activity in mice and tissue cultures. This latter project is presently being supported by The Council For Tobacco Research-U.S.A.

Signature John C. Manos, M.D.
Director of Project

[signature]
Business Officer of the Institution

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